CARDIOVASCULAR MEDICINE

Long term efficacy and safety of atorvastatin in the treatment of severe type III and combined dyslipidaemia

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Background: Fibric acid derivatives and HMG-CoA reductase inhibitors are effective in combination for treating patients with familial dysbetalipoproteinaemia and severe combined dyslipidaemia, but combination therapy affects compliance and increases the risk of side effects.

Aim: To evaluate the efficacy and safety of monotherapy with atorvastatin, an HMG-CoA reductase inhibitor with superior efficacy in lowering low density lipoprotein cholesterol and triglyceride concentrations, in patients with dysbetalipoproteinaemia and severe combined dyslipidaemia.

Methods: Atorvastatin was tested as single drug treatment in 36 patients with familial dysbetalipoproteinaemia and 23 patients with severe combined dyslipidaemia.

Results: After 40 weeks of 40 mg atorvastatin treatment decreases in total cholesterol, triglycerides, and apolipoprotein B of 40%, 43%, and 41%, respectively, were observed in the combined dyslipidaemia group, and of 46%, 40%, and 43% in the dysbetalipoproteinaemic patients. Target concentrations of total cholesterol (< 5 mmol/l) were reached by 63% of the patients, and target concentrations of triglycerides (< 3.0 mmol/l) by 66%. Treatment with atorvastatin was well tolerated and no serious side effects were reported.

Conclusions: Atorvastatin is very effective as monotherapy in the treatment of familial dysbetalipoproteinaemia and severe combined dyslipidaemia.

Type III dyslipidaemia or familial dysbetalipoproteinaemia (FD) is a severe disturbance of plasma lipoprotein metabolism associated with premature vascular disease. FD is associated with raised concentrations of both cholesterol and triglycerides because of an accumulation of cholesterol ester enriched chylomicron and very low density lipoprotein (VLDL) remnants (IDL cholesterol).[1] FD has a recessive mode of inheritance, a frequency of approximately 1:10 000, and is usually manifested in adulthood. The disease is far more prevalent in men than in women, who usually only present with this disorder after the menopause.[1]

Almost half the patients with FD have symptoms of premature cardiovascular disease. The distribution of vascular disease differs from that in other genetic lipoprotein disorders, as peripheral vascular disease is as prevalent as coronary artery disease.[1 2] The metabolic basis of the accumulation of IDL lies in the impaired clearance of these particles by hepatic lipoprotein receptors. Apolipoprotein E (apo E) is the major ligand for these receptors and mediates chylomicron and VLDL remnant uptake.[3–6] Apo E is a 299 amino acid protein and a major constituent of plasma lipoproteins such as chylomicrons, chylomicron remnants, VLDL, IDL, and high density lipoprotein (HDL).[5] Apo E has three major isoforms (E2, E3, and E4). These are all products of alleles at a single gene locus on chromosome 19.[7–9] Apo E3/E3 is the most common genotype and therefore E2 and E4 are considered variants. The apo E3 isoform shows normal binding to the low density lipoprotein (LDL) receptor, while apo E4 binds with a somewhat higher affinity and the apo E2 isoform shows very low affinity.[10 11] FD occurs most commonly in association with the apo E2/E2 genotype (> 90%).[12–15] However, several rare apo E mutants also lead to dysbetalipoproteinaemia, but usually with a dominant expression (for example, apo E3-Leiden, apo E2 (Lys146→Gln)).[16–19]

There is a large discrepancy between the occurrence of homozygous apo E2 (1:100) in the general population and the prevalence of FD (1:10 000). Most E2/E2 carriers are either normolipidaemic or even slightly hypocholesterolaemic.[1] Other genetic or environmental factors such as a high alcohol intake, obesity, or concomitant diseases (for example, diabetes mellitus or thyroid dysfunction) may be required for the development of the FD phenotype.

Patients with FD have a high risk of premature cardiovascular disease and should thus be treated aggressively.[3 6] Fortunately, FD often shows a favourable response to lipid lowering treatment.[1] First line treatment is dietary control and weight reduction. However, drugs are often required to control raised concentrations of plasma lipoproteins. Several lipid lowering agents can be given to FD patients, including fibric acid derivates and HMG-CoA reductase inhibitors.[20–34] Often, a combination of these drugs is necessary to normalise both cholesterol and triglyceride concentrations.

Although the use of HMG-CoA reductase inhibitors has mainly been directed at decreasing LDL cholesterol, over the last few years the triglyceride lowering effect of these agents has aroused interest. Recently, it has been shown that statins reduce triglycerides by almost the same percentage as they do LDL cholesterol in hypertriglyceridaemic subjects.[35–37] Atorvastatin, an established third generation statin with proven effectiveness in reducing plasma LDL cholesterol and triglyceride concentrations, could thus be well suited to treating patients with FD.[38–42]

In this study we evaluated the efficacy and safety of atorvastatin monotherapy in outpatients with severe combined dyslipidaemia, among whom a subset had molecularly diagnosed dysbetalipoproteinaemia.

Abbreviations: apo E, apolipoprotein E; C, cholesterol; FD, familial dysbetalipoproteinaemia; HDL, high density lipoprotein; IDL, intermediate density lipoprotein (VLDL remnants); LDL, low density lipoprotein; TG, triglyceride; VLDL, very low density lipoprotein.

Table 1 Baseline characteristics

Demographics		
Total number of patients		59
Age (years) (range)		51.5 (28 to 79)
Male/female (%)		43 (73)/16 (27)
Postmenopausal status (%)		16.7
History		
Cardiovascular disease (%)		28.8
Smoking (%)		30.5
Hypertension (%)		15.3
Diabetes mellitus (%)		10.2
Alcohol use (%)	≤2 units a day	80.5
	>2 units a day	6.7
Vital signs		
Body mass index (kg/m^2)*		27.2 (4.6)
Systolic blood pressure (mm Hg)*		128 (10.5)
Diastolic blood pressure (mm Hg)*		81.5 (5.4)
Apolipoprotein E genotype†		
E2/E2		34
E2 (lys146→gln)		2
E2/E3		16
E2/E4		7

*Values are given as mean (SD); †apo E genotypes are given in absolute numbers.

METHODS
Patients
We recruited consecutive patients with a tentative diagnosis of dysbetalipoproteinaemia at the lipid research clinic of the University of Amsterdam (Academic Medical Centre and Slotervaart Training Hospital) by retrieving patient records from our clinical database. Eligible patients were men and women between 18 and 80 years of age with a diagnosis of probable dysbetalipoproteinaemia. This diagnosis was made when a patient presented with severe combined dyslipidaemia (both total cholesterol and triglyceride concentrations significantly above the 95% centile for age and sex), occasionally typical xanthomas, and in many cases atherosclerotic vascular disease. All eligible patients were informed about atorvastatin and asked to participate in the study. Patients with poorly controlled diabetes mellitus, hypothyroidism, and hepatic or renal dysfunction were excluded. Pregnant or breast feeding women were also excluded.

Before entry to the study and at its completion, all patients underwent a complete physical examination and were counselled on the use of a lipid lowering diet, according to the Dutch national guidelines. Written informed consent was obtained from all patients.

Study design
At the start of the study all lipid lowering drugs were discontinued for eight weeks. After this, baseline lipids, apolipoproteins, and apo E genotyping were undertaken in all patients, together with routine biochemistry. Upon confirmation of homozygosity for apo E2 (arg 158→cys) (E2/E2) or the presence of the apo E2 variant (lys 146→gln), lipoprotein ultracentrifugation was performed. Subsequently, atorvastatin was prescribed, and patients were examined at regular 12 week intervals, concluding at week 40.

At all visits, the patients were questioned about adherence to the diet and possible side effects of the drug treatment. They then had a physical examination. At each visit, blood samples were taken after an overnight fast for the determination of plasma lipids, apolipoproteins, and safety indices. These measurements were done at the central laboratory of both hospitals in Amsterdam. Extra blood samples were drawn from apo E2/E2 subjects or heterozygous carriers of the apo E2 (lys 146→gln) variant to assess the efficacy of atorvastatin towards VLDL, IDL, LDL, and HDL cholesterol and towards VLDL and LDL triglycerides. These ultracentrifugation samples were taken at baseline and at the end of the study (after
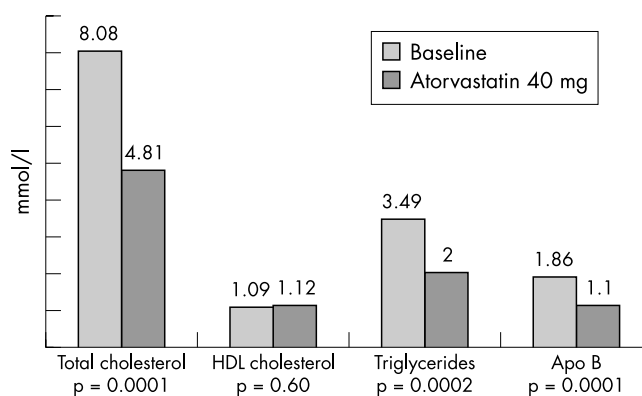
Figure 1 Lipoprotein and apolipoprotein concentrations before and after atorvastatin treatment in patients with combined dyslipidaemia. Mean values are given; triglyceride values were log transformed before statistical analysis.

40 weeks of treatment) and were analysed at TNO-PG, Gaubius Laboratory, Leiden. After completion of the study we compared our results with those of other pharmacological studies already performed in patients with FD and to previous lipid results in our patient cohort.

Lipid and apolipoprotein measurements
Total plasma cholesterol was determined by an enzymatic colorimetric procedure using cholesterol esterase and cholesterol oxidase (CHOD-PAP, Boehringer Mannheim, Mannheim, Germany). HDL cholesterol was determined after precipitation of apo B containing lipoproteins, using phosphotungstic acid and magnesium ions. Triglycerides were quantified by an enzymatic colorimetric procedure using lipase, glycerokinase, and glycerol-3-phosphate oxidase. Apo A1 and apo B were measured by immunonephelometry.

Lipoprotein analysis
Blood samples were collected in EDTA tubes and placed on melting ice. Plasma was obtained by centrifugation at 1500 ×g for 10 minutes at 4°C within four hours after sampling. Plasma samples were then brought to a final concentration of 10% (wt/vol) sucrose, capped under nitrogen, snap frozen in liquid nitrogen, and stored at −80°C until further analysis. Under these conditions, lipoprotein size and biological properties have been shown to remain intact for months.[43]

Separation of lipoproteins was done by density gradient ultracentrifugation according to Zhao and colleagues,[44] with slight modifications. Briefly, the gradient consisted of 2 ml plasma (adjusted to d = 1.21 g/ml by adding 0.65 g KBr), overlayered by 5 ml of d = 1.03 g/ml, 3.5 ml of d = 1.006 g/ml, and 1.5 ml of d = 1.00 g/ml solutions. The gradient was centrifuged at 285 000 ×g in a SW-40 swingout rotor (Beckman, Geneva, Switzerland) for 18 hours at 4°C. The gradient was then divided into fractions of 0.5 ml. In each fraction, cholesterol and triglyceride concentrations were measured with enzymatic assay kits (Boehringer Mannheim).

Apolipoprotein E phenotyping and genotyping
Homozygosity for the apo E2 (arg 158→cys) isoform was determined by isoelectric focusing of delipidated serum samples followed by immunoblotting with a polyclonal anti-apo E antiserum,[45] and confirmed by apo E genotyping as previously described by Reymer and colleagues.[46] Identification of the apo E2 variant (lys 146→gln) was by polymerase chain reaction using a mutagenic amplification primer assay followed by digestion with restriction enzyme *Pvu* II as described previously.[18]

Statistical analysis
The analyses, which were done using SAS 6.1.2, addressed the change in lipid and apolipoprotein variables and lipoprotein
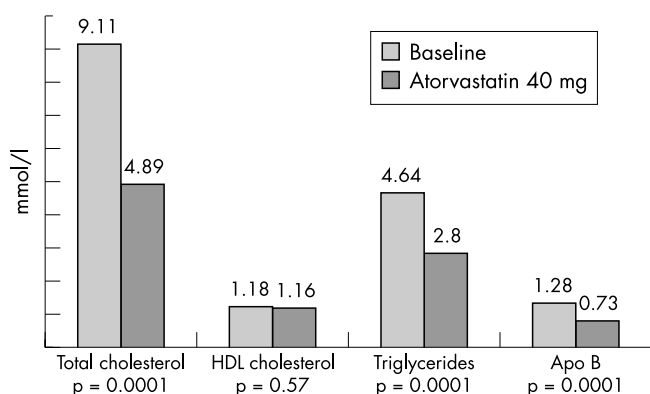
Figure 2 Lipoprotein and apolipoprotein concentrations before and after atorvastatin treatment in patients with dysbetalipoproteinaemia. Mean values are given; triglyceride values were log transformed before statistical analysis.

subfractions before and after atorvastatin treatment. Before analysis, triglyceride, VLDL-C, VLDL-TG, and IDL-TG values were logarithmically transformed. Untransformed concentrations are reported in the tables. Significance was assessed at the 5% level of probability.

RESULTS
Patient characteristics
Fifty nine patients (43 men and 16 women) were included in the study. Clinical and biochemical baseline characteristics are summarised in table 1. The mean age of the patients was 51.5 years (range 28–79 years) and mean (SD) body mass index was 27.2 (4.6) kg/m . Most patients included in the study were of north European descent (97%).

Thirty four patients were homozygous for the apo E2 genotype and two patients were heterozygous carriers of the apo E2 (lys 146→gln) variant. The remaining 23 patients either had an apo E2/E3 or an apo E2/E4 genotype. Baseline lipoprotein profiles of the two groups are shown in figs 1 and 2.

Mean plasma lipid concentrations in both groups were notably raised. Mean (SD) total cholesterol and median triglyceride concentrations were 8.1 (2.3) mmol/l and 3.5 (1.7) mmol/l, respectively, in the group with severe combined dyslipidaemia. In the group with dysbetalipoproteinaemia the baseline values were 9.1 (2.8) mmol/l for total cholesterol and 4.6 (2.0) mmol/l for triglycerides. Baseline lipoprotein subfractionation could be done in 28 patients with an apo E2/E2 genotype. Seventeen had established cardiovascular disease—that is, angina pectoris, coronary atherosclerosis at coronary angiography, a positive stress test, previous myocardial infarction, or a revascularisation procedure. Six patients had well controlled diabetes mellitus (one was on insulin and the others on oral antidiabetic treatment). Twenty patients were

Table 3 Safety indices before and after atorvastatin treatment in patients with dysbetalipoproteinaemia and combined dyslipidaemia

	n	Baseline	Atorvastatin 40 mg
CPK (U/l)	50	97 (73)	86 (52)
ALAT (U/l)	54	23 (16)	24 (19)
ASAT (U/l)	54	19 (11)	15 (8)

Values are mean (SD).
ALAT, alanine aminotransferase; ASAT, asparginine aminotransferase; CPK, creatinine phosphokinase.

known to be taking one or more drugs that might affect lipid metabolism, including β blocking agents (n = 18), diuretics (n = 6), and oral contraceptives (n = 7). These drugs were prescribed continuously during the course of the study. None of the postmenopausal women who were participating were taking hormone replacement therapy.

Fifty patients completed the study. Five patients dropped out because of adverse events: three patients had gastric complaints (all E2/E3 or E2/E4), one suffered from psychological problems (E2/E2), and one developed viral hepatitis (E2/E2).

Four patients (all E2/E3 or E2/E4) dropped out because of non-compliance.

Efficacy
Lipids and lipoproteins
At 40 weeks of treatment, the efficacy of atorvastatin 40 mg was examined in both patient groups (figs 1 and 2). A significant decrease in total cholesterol, triglycerides, and apo B (by 40%, 43%, and 41%, respectively) was observed in the combined dyslipidaemia group (fig 1); all reductions were highly significant (p ⩽ 0.0002). Reductions were also seen for total cholesterol, triglycerides, and apo B concentrations (by 46%, 40%, and 43%, respectively; p = 0.0001) in patients with dysbetalipoproteinaemia (fig 2). Target concentrations of total cholesterol (< 5 mmol/l) were reached by 63% of patients, and target concentrations of triglycerides (< 3 mmol/l) by 66%.

Lipoprotein subfractions
In patients with homozygosity for apo E2 and heterozygous carriers of the apo E2 (lys 146→gln) variant, the efficacy of atorvastatin on lipoprotein subfractions was examined at baseline and after 40 weeks. A significant decrease was observed in almost all lipoprotein subfractions (table 2). For LDL-C, VLDL-C, and IDL-C these reductions were 38%, 59%, and 51%, respectively (p = 0.0001). The VLDL-C to triglycerides ratio, an important marker of dysbetalipoproteinaemia, decreased from 1.15 at baseline to 0.79 after 40 weeks of atorvastatin treatment.

Table 2 Lipoprotein subfractions before and after atorvastatin treatment in patients with dysbetalipoproteinaemia

	Baseline (n=28)	Atorvastatin 40 mg (n=28)	p Value
VLDL-C* (mmol/l)	4.24 (3.39)	1.72 (1.21)	0.0001
IDL-C (mmol/l)	1.80 (0.80)	0.89 (0.38)	0.0001
LDL-C (mmol/l)	1.87 (0.56)	1.16 (0.47)	0.0001
HDL-C (mmol/l)	1.08 (0.28)	1.19 (0.32)	0.06
VLDL-TG* (mmol/l)	4.08 (4.00)	2.21 (1.70)	0.0014
IDL-TG* (mmol/l)	0.59 (0.24)	0.32 (0.14)	0.0001
VLDL-C/TG (ratio)	1.15 (0.32)	0.79 (0.32)	0.0001

Values are mean (SD).
*VLDL-C, VLDL-TG, and IDL-TG values were log transformed before statistical analysis.
IDL-C, intermediate density lipoprotein cholesterol; IDL-TG, intermediate density lipoprotein triglycerides; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; VLDL-TG, very low density lipoprotein triglycerides.

Table 4 Published reports on lipid lowering treatment in familial dysbetalipo-proteinaemia

	Percentage change with drug treatment				
	Gemfibrozil[22] (n=12)	Fenofibrate[29] (n=9)	Lovastatin[20] (n=12)	Simvastatin[21] (n=19)	Atorvastatin (n=28)
Total cholesterol (mmol/l)	48	35	44	39	46
HDL-C (mmol/l)	+13	+33	+25	+18	+10
Triglycerides (mmol/l)	68	56	42	41	40
VLDL-C (mmol/l)	72	63	53	44	59

HDL-C, high density lipoprotein cholesterol; VLDL-C, very low density lipoprotein cholesterol.

Side effects and tolerance

Treatment with atorvastatin was well tolerated. Eleven of the 59 patients reported side effects that could possibly be related to the treatment. Three suffered from gastric complaints, another four had joint or muscle complaints, and two had a rash, though in one of these it was probably related to moisturising products. One patient suffered from impotence. The three patients with gastric complaints all dropped out, as did the patient with psychological problems and the patient with viral hepatitis.

Other reported side effects, all occurring during the first eight weeks of treatment, disappeared during the course of the study. Clinical laboratory changes were minimal in both study groups. There was no clinically significant increase in creatinine phosphokinase activity (that is, to more than three times the upper limit of normal (193 U/l)). Only γ-glutamyl transferase (γ-GT) showed mild increases in four patients after the first eight weeks of treatment. These disappeared during the study. In one subject the γ-GT value exceeded three times the upper limit of normal (58 U/l) and remained high, most probably because of a high alcohol intake. Mean baseline and 40 week concentrations of alanine aminotransferase, asparginine aminotransferase, and creatine phosphokinase are given in table 3. None of the differences reached significance.

DISCUSSION

We have studied the efficacy and safety of atorvastatin in patients with severe combined dyslipidaemia and dysbetalipoproteinaemia. Our findings show that this drug is a very effective cholesterol and triglyceride lowering agent in the treatment of both groups of patients. Target concentrations of total cholesterol (< 5 mmol/l) were reached in 63% of patients, and of triglycerides (< 3 mmol/l) in 66% of patients.

FD comprises a separate nosological entity in the spectrum of severe combined dyslipidaemia, with an extremely atherogenic lipoprotein profile and therefore a high risk of premature atherosclerosis. It should be managed aggressively with lipid lowering treatment and is usually responsive to such interventions.[1] First line treatment should be diet and lifestyle adjustments, followed by the correction of pre-existing metabolic disorders. In the majority of FD patients, however, lipid lowering drugs are required to control dyslipidaemia. Drugs with proven efficacy in FD include nicotinic acid,[25 26] clofibrate,[20 25–27] fenofibrate,[28 29 32] gemfibrozil,[26 30 31 33] lovastatin,[20 27 34] and simvastatin.[21 23] Unfortunately, many patients remain hyperlipidaemic on diet and single drug treatment and often require combination treatment with a fibric acid derivate and a statin. However, it is well established that combination treatment affects compliance and increases the risk of side effects such as liver dysfunction or myopathy.[47]

Atorvastatin is an established and effective HMG-CoA reductase inhibitor. Clinical studies with this agent have shown that LDL cholesterol concentrations may be decreased by up to 61% at doses of 80 mg, and triglycerides may be reduced by 46%.[38–40] Furthermore, it has been found recently that atorvastatin reduces both VLDL and LDL apo B production and in addition increases the clearance of triglyceride-rich lipoproteins.[41 42]

McKenny and colleagues recently reported that atorvastatin is very effective as monotherapy in lowering both cholesterol and triglycerides in patients with combined dyslipidemia.[48] In this study, we addressed the question of whether atorvastatin might also be a good candidate as monotherapy in 23 patients with severe combined dyslipidaemia and 36 patients with molecularly diagnosed dysbetalipoproteinaemia. In keeping with the results of McKenny and colleagues, atorvastatin showed good efficacy and safety in our patient cohort. In the group with severe combined dyslipidaemia, total cholesterol, triglycerides, and apo B decreased by 40%, 43%, and 41%, respectively, while in the patients with dysbetalipoproteinaemia the decreases were 46%, 40% and 43%. Furthermore, VLDL-C and VLDL-TG fell by 59% and 46%, respectively, and IDL-C and IDL-TG by 51% and 46% compared with baseline. The VLDL-C/TG ratio fell from 1.15 to 0.79.

Data on the efficacy of lipid lowering drugs in the treatment of FD are only available for small numbers of patients. The confidence intervals in those studies were large, making it difficult to draw firm conclusions about differences in various therapeutic regimens (table 4). Treatment of FD patients with fibrates generally results in a decrease in total cholesterol of up to 48%, and an increase in HDL of up to 33%. Triglyceride concentrations were decreased by up to 68%. IDL, as a marker of FD, decreased by 34% when treatment with gemfibrozil was initiated. In the treatment of FD with statins, total cholesterol was decreased by up to 46% and HDL was raised by up to 25%. Triglycerides were decreased by up to 42%. IDL showed a reduction of 50% after treatment with simvastatin 20 mg.

We investigated the efficacy of atorvastatin in 36 FD patients, a substantially larger population than previously reported. Moreover these patients were followed for a longer period of time than in most other studies. However, a definite disadvantage of our study is the lack of a control or placebo group. The use of placebo groups in treatment studies of genetic dyslipidaemia is no longer considered ethical, and the relatively small number of FD patients precluded the use of a formal double blind control comparison. We have, however, compared baseline and 40 week lipid and lipoprotein concentrations and have shown highly significant and impressive reductions on 40 mg atorvastatin monotherapy. Side effects were mild and generally transient, as reported for other studies with this drug.

Conclusions

Atorvastatin proved to be both effective and safe when given as monotherapy in the treatment of severe combined dyslipidaemia and familial dysbetalipoproteinaemia.

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REFERENCES

1 **Mahley RW**, Rall SC. Type III hyperlipidemia (dysbetalipoproteinemia). The role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In: Scriver CR, Beaudet AL, Sly WS, eds. *The metabolic basis of inherited disease.* New York: McGraw-Hill, 1989:1195–213.
2 **Cabin HS**, Schwartz DE, Virmani R, et al. Type III hyperlipoproteinemia: quantification, distribution and nature of atherosclerotic coronary arterial narrowing in five necropsy patients. *Am Heart J* 1981;**102**:830–5.
3 **Rall SC**, Mahley RW. The role of apolipoprotein E genetic variants in lipoprotein disorders. *J Intern Med* 1992;**231**:653–9.
4 **Utermann G**, Vogelberg KH, Steinmetz A, et al. Polymorphism of apolipoprotein E. II. Genetics of hyperlipoproteinemia type III. *Clin Genet* 1979;**15**:37–62.
5 **Rall SC**, Weisgraber KH, Mahley RW. Human apolipoprotein E; the complete amino acid sequence. *J Biol Chem* 1982;**257**:4171–8.
6 **Mahley RW**. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 1988;**240**:622–30.
7 **Utermann G**, Steinmetz A, Weber W. Genetic control of human apolipoprotein E polymorphism: comparison of one- and two-dimensional techniques of isoprotein analysis. *Hum Genet* 1982;**60**:344–51.
8 **Utermann G**, Hees M, Steinmetz A. Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinemia in man. *Nature* 1977;**269**:604–7.
9 **Zannis VI**, Breslow JL. Human very low density lipoprotein apolipoprotein E isoprotein polymorphism is explained by genetic variation and post-translational modification. *Biochemistry* 1981;**20**:1033–41.
10 **Schneider WJ**, Kovanan PT, Brown MS, et al. Familial dysbetalipoproteinemia: abnormal binding of mutant apolipoprotein E to low-density lipoprotein receptors of human fibroblasts and membranes from liver and adrenals of rats, rabbits and cows. *J Clin Invest* 1981;**68**:1075–85.
11 **Davignon J**, Gregg RE, Sing CF. Apolipoprotein E: polymorphism and atherosclerosis. *Atherosclerosis* 1988;**8**:1–21.
12 **Utermann G**, Jaeschke M, Menzel J. Familial hyperlipidemia type III: deficiency of a specific apolipoprotein (Apo E III) in the very low-density lipoproteins. *FEBS Lett* 1975;**56**:352–5.
13 **Utermann G**, Vogelberg KH, Steinmetz A, et al. Polymorphism of apolipoprotein E. II. Genetics of hyperlipoproteinemia type III. *Clin Genet* 1979;**15**:37–62.
14 **Zannis VI**, Breslow JL. Characterization of a unique human apolipoprotein E variant, associated with type III hyperlipoproteinemia. *J Biol Chem* 1980;**255**:1759–62.
15 **Mahley RW**, Weisgraber KH, Innerarity TL, et al. Genetic defects in lipoprotein metabolism: elevation of atherogenic lipoproteins caused by impaired catabolism. *JAMA* 1991;**265**:78–83.
16 **Stuyt PM**, Demacker PN, van't Laar A. Serum lipids, lipoproteins and apolipoprotein E phenotypes in relatives of patients with type III hyperlipidemia. *Eur J Clin Invest* 1984;**14**:219–26.
17 **De Knijff P**, Van den Maagdenberg AM, Stalenhoef AF, et al. Familial dysbetalipoproteinemia associated with apolipoprotein E3-Leiden in an extended multigeneration pedigree. *J Clin Invest* 1991;**88**:643–55.
18 **De Knijff P**, Van den Maagdenberg AM, Boomsma DI, et al. Variable expression of familial dysbetalipoproteinemia in apolipoprotein E*2 (Lys146→Gln) allele carriers. *J Clin Invest* 1994;**94**:1252–62.
19 **Civeira F**, Pocovi M, Cenarro A, et al. Apo E variants in patients with type III hyperlipoproteinemia. *Atherosclerosis* 1996;**127**:273–82.
20 **Illingworth DR**, O'Malley JP. The hypolipidemic effects of lovastatin and clofibrate alone and in combination in patients with type III hyperlipoproteinemia. *Metabolism* 1990;**39**:403–9.
21 **Feussner G**, Eichinger M, Ziegler R. The influence of simvastatin alone or in combination with gemfibrozil on plasma lipids and lipoproteins in patients with type III hyperlipidemia. *Clin Invest* 1992;**70**:1027–35.
22 **Larsen ML**, Illingworth DR, O'Malley JP. Comparative effects of gemfibrozil and clofibrate in type III hyperlipoproteinemia. *Atherosclerosis* 1994;**106**:235–40.
23 **Zhao S**, Smelt AH, Van den Maagdenberg AM, et al. Plasma lipoproteins in familial dysbetalipoproteinemia associated with apolipoproteins E2 (Arg158→Cys), E3-Leiden, and E2(Lys146→Gln), and effects of treatment of simvastatin. *Arterioscler Thromb* 1994;**14**:1705–16.
24 **Zhao S**, Smelt AH, Leuven JA, et al. Changes of lipoprotein profile in familial dysbetalipoproteinemia with gemfibrozil. *Am J Med* 1994;**96**:49–56.
25 **Patsch JR**, Yeshurun D, Jackson RL, et al. Effects of clofibrate, nicotinic acid and diet on the properties of the plasma lipoproteins in a subject with type III hyperlipoproteinemia. *Am J Med* 1977;**63**:1001–9.
26 **Hoogwerf BJ**, Bantle JP, Kuba K, et al. Treatment of type III hyperlipoproteinemia with four different treatment regiments. *Atherosclerosis* 1984;**51**:251–9.
27 **Falko JM**, Witztum JL, Schonfeld G, et al. Type III hyperlipoproteinemia: rise in high-density lipoprotein levels in response to therapy. *Am J Med* 1979;**66**:303–10.
28 **Lussier-Cacan S**, Bard JM, Boelet L, et al. Lipoprotein composition changes induced by fenofibrate in dysbetalipoproteinemia type III. *Atherosclerosis* 1989;**78**:167–82.
29 **Fruchart JC**, Davignon J, Bard JM, et al. Effect of fenofibrate treatment on type III hyperlipoproteinemia. *Am J Med* 1987;**83**(suppl 5B):71–4.
30 **Kuo PT**, Wilson AC, Kostis JB, et al. Treatment of type III hyperlipoproteinemia with gemfibrozil to retard progression of coronary artery disease. *Am Heart J* 1988;**116**:85–90.
31 **Houlston R**, Quiney J, Watts GF, et al. Gemfibrozil in the treatment of resistant familial hypercholesterolemia and type III hyperlipoproteinemia. *J R Soc Med* 1988;**81**:274–6.
32 **Otto C**, Ritter MM, Soennichsen AC, et al. Effects of n-3 fatty acids and fenofibrate on lipid and hemorrheological parameters in familial dysbetalipoproteinemia and familial hypertriglyceridemia. *Metabolism* 1996;**45**:1305–11.
33 **East CA**, Grundy SM, Bilheimer DW. Preliminary report: treatment of type III hyperlipoproteinemia with mevinolin. *Metabolism* 1996;**35**:97–8.
34 **Vega GL**, East C, Grundy SM. Lovastatin therapy in familial dysbetalipoproteinemia: effects on kinetics of apolipoprotein B. *Atherosclerosis* 1988;**70**:131–43.
35 **Grundy SM**. Consensus statement: role of therapy with "statins" in patients with hypertriglyceridemia. *Am J Cardiol* 1998;**81**:1–6B.
36 **Ginsberg HN**. Effects of statins on triglyceride metabolism. *Am J Cardiol* 1998;**81**:32–5B.
37 **Stein EA**, Lane M, Laskarzewski P. Comparison of statins in hypertriglyceridemia. *Am J Cardiol* 1998;**81**:66–9B.
38 **Nawrocki JW**, Weiss SR, Davidson MH, et al. Reduction of LDL cholesterol by 25% to 60% in patients with primary hypercholesterolemia by atorvastatin, a new HMG-CoA reductase inhibitor. *Arterioscler Thromb Vasc Biol* 1995;**15**:678–82.
39 **Bakker-Arkema RG**, Davidson MH, Goldstein RJ, et al. Efficacy and safety of a new HMG-CoA reductase inhibitor, atorvastatin, in patients with hypertriglyceridemia. *JAMA* 1996;**275**:128–33.
40 **Alaupovic P**, Heinonen T, Schurzinske L, et al. Effect of a new HMG-CoA reductase inhibitor, atorvastatin, on lipids, apolipoproteins and lipoprotein particles in patients with elevated serum cholesterol and triglyceride levels. *Atherosclerosis* 1997;**133**:123–33.
41 **Burnett JR**, Wilcox LJ, Telford DE, et al. Inhibition of HMG-CoA reductase by atorvastatin decreases both VLDL and LDL apolipoprotein B production in miniature pigs. *Arterioscler Thromb Vasc Biol* 1997;**17**:2589–600.
42 **Burnett JR**, Barrett PH, Vicini P, et al. The HMG-CoA reductase inhibitor atorvastatin increases the fractional clearance rate of postprandial triglyceride-rich lipoproteins in miniature pigs. *Arterioscler Thromb Vasc Biol* 1998;**18**:1906–14.
43 **Rumsey SC**, Galeano NF, Arad Y, et al. Cryopreservation with sucrose maintains normal physical and biological properties of human plasma low-density lipoproteins. *J Lipid Res* 1992;**33**:1551–61.
44 **Zhao SP**, Bastiaanse EM, Hau MF, et al. Separation of VLDL subfractions by density gradient ultracentrifugation. *J Lab Clin Med* 1995;**125**:641–9.
45 **Havekes LM**, de Knijff P, Beisiegel U, et al. A rapid micromethod for apolipoprotein E phenotyping directly in serum. *J Lipid Res* 1987;**28**:455–63.
46 **Reymer PW**, Groenemeyer BE, van de Burg R, et al. Apolipoprotein E genotyping on agarose gels. *Clin Chem* 1995;**41**:1046–7.
47 **Blackwell B**. Drug therapy: patient compliance. *N Engl J Med* 1973;**289**:249–52.
48 **McKenney JM**, McCormick LS, Weiss S, et al. A randomized trial of the effects of atorvastatin and niacin in patients with combined hyperlipidemia or isolated hypertriglyceridemia. Collaborative Atorvastatin Study Group. *Am J Med* 1998;**104**:137–43.